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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 9/10, 9/64, C12P 21/06	A1	(11) International Publication Number: **WO 96/40881** (43) International Publication Date: 19 December 1996 (19.12.96)

(21) International Application Number: PCT/US96/06427

(22) International Filing Date: 8 May 1996 (08.05.96)

(30) Priority Data:
08/483,151 7 June 1995 (07.06.95) US

(71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; 55 Fruit Street, Boston, MA 92114 (US).

(72) Inventors: SEED, Brian; Nine Hawthorne Place #5J, Boston, MA 02114 (US). HOLGERSSON, Jan; Fatburs Kvarngata 5, S-118 64 Stockholm (SE).

(74) Agent: LECH, Karen, F.; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110-2804 (US).

(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: FUCOSYLTRANSFERASE GENES AND USES THEREOF

(57) Abstract

Purified DNA encoding α(1,3)fucosyltransferase and the recombinant proteins expressed from such DNA are disclosed. The recombinant fucosyltransferase polypeptides are used to fucosylate proteins to produce therapeutics useful for the treatment of disease, e.g., an adverse immune reaction such as septicemia or septic shock.

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FUCOSYLTRANSFERASE GENES AND USES THEREOF

Statement as to Federally Sponsored Research

This invention was made at least in part with funds from the Federal Government, and the Government therefore has certain rights in the invention.

Background of the Invention

This invention relates to recombinant fucosyltransferases, DNA, and uses thereof.

The structurally related endothelial cell receptors E-selectin (Bevilacqua et al., *Proc. Natl. Acad. Sci. USA* 84:9238, 1987; Bevilacqua et al., *Science* 243:1160, 1989) and P-selectin (Hsu-Lin et al., *J. Biol. Chem.* 259:9121, 1984; Stenberg et al., *J. Cell Biol.* 101:880, 1985; Johnston et al., *Blood* 69:1401, 1987) mediate myeloid cell attachment to the vascular wall following activation of the endothelial cell by inflammatory cytokines (in the case of E-selectin; see, e.g., Bevilacqua et al. (1987), supra) or thrombin (in the case of P-selectin; see, e.g., Geng et al., *Nature* 343:757, 1990). Expressed both by Weibel-Palade bodies of endothelium (McEver et al., *J. Clin. Invest.* 84:920, 1989; Bonfanti et al., *Blood* 73:1109, 1989) and platelet alpha granules (Hsu-Lin et al., supra; Stenberg et al., supra), P-selectin also mediates monocyte and neutrophil binding to activated platelets (Larsen et al., *Cell* 59:305, 1989; Hamburger et al., *Blood* 75:550, 1990). The leading candidate ligands for the two receptors are the sialyl-Le^x structure for E-selectin (Lowe et al., *Cell* 63:475, 1990; Phillips et al., *Science* 250:1130, 1990); Walz et al., *Science* 250:1132, 1990) and the same glycan in the context of a specific mucin, PSGL-1, (P-selectin glycoprotein ligand 1) for P-selectin (Sako et al., *Cell* 75:1179, 1993).

Genetic and biochemical data have demonstrated the existence of at least four distinct types of fucosyltransferases capable of forming the α(1,4) linkage: the Lewis enzyme (Fuc-TIII), which can transfer fucose either α(1,3) or α(1,4) to Galβ4GlcNAc or Galβ3GlcNAc respectively (Kukowska-Latallo et al., *Genes Dev.* 4:1288, 1990); at least one enzyme, Fuc-TIV, solely forming α(1,3) linkages, which cannot utilize sialylated substrates (Goelz et al., *Cell* 63:1349, 1989; Lowe et al., *J. Biol. Chem.* 266:17467, 1991); at least two enzymes, Fuc-TV (Weston et al., *J. Biol. Chem.* 267:4152, 1992a) and Fuc-TVI (Weston et al., *J. Biol. Chem.* 267:24575, 1992b) solely forming α(1,3) linkages, which can fucosylate either sialylated or nonsialylated precursors, and a recently described enzyme, Fuc-TVII, (Sasaki et al., *J. Biol. Chem.* 269:14730, 1994); Natsuka et al., *J. Biol. Chem.* 269:16789, 1994) which can fucosylate only sialylated precursors.

Summary of the Invention

In general, the invention features substantially pure α(1,3) fucosyltransferase, including an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2). In preferred embodiments, pure α(1,3) fucosyltransferase is obtained from a mammal (for example, a murine cell line (e.g., 32D cl3), or from a human).

In a related aspect, the invention features a fragment or analog of α(1,3)fucosyltransferase polypeptide including an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2).

In another related aspect, the invention features substantially pure DNA having a sequence substantially identical to the nucleotide sequence shown in Fig. 3 (SEQ

ID NO: 1). In preferred embodiments, such DNA is cDNA or is genomic DNA. In related aspects, the invention also features a vector and a cell (e.g., a murine cell such as 32D c13 or a human cell such as human cell line 293) which includes such substantially pure DNA. In various preferred embodiments, the vector-containing cell is a prokaryotic cell, for example, E. coli, or, more preferably, is a eukaryotic mammalian cell (e.g., the murine cell line 32D c13 or human cell line 293).

In yet another related aspect, the invention features a method of fucosylating a polypeptide in vivo involving: (a) providing a cell containing the fucosyltransferase DNA of the invention including a nucleotide sequence which is substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 1) positioned for expression in the cell; and (b) culturing the transformed cell under conditions for expressing the DNA, resulting in the fucosylation of the protein.

In preferred embodiments, fucosylation occurs in a mammalian cell, for example, a human cell (e.g., human cell line 293) or a murine cell (e.g., 32D c13). In related aspects, the cell contains a second fucosyltransferase gene. Preferably, such a second gene is substantially identical to the nucleotide sequence shown in Fig. 6A (SEQ ID NO: 3) which encodes a polypeptide including an amino acid sequence substantially identical to the sequence shown in Fig. 6B (SEQ ID NO: 4). In preferred embodiments, the protein which is fucosylated according to the above method is an AGP-antibody fusion protein or is an antibody (e.g., IgG or IgM).

In another aspect, the invention features a recombinant polypeptide fucosylated using a cell expressing DNA which is substantially identical to the nucleotide sequence shown in Fig. 3 (SEQ ID NO: 1).

Preferably, the fucosylated polypeptide is an AGP-antibody fusion protein, or is an antibody (e.g., IgG or IgM). In still other preferred embodiments, the polypeptide is further fucosylated using a second fucosyltransferase. Such a second fucosyltransferase is substantially identical to a polypeptide including an amino acid sequence shown in Fig. 6B (SEQ ID NO: 4). Preferably, the cell used to fucosylate the polypeptide is a mammalian cell (e.g., the murine cell line 32D c13 or the human cell line 293).

In another aspect, the invention features a polypeptide fucosylated in vitro using a fucosyltransferase having an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2). In preferred embodiments, the fucosylated polypeptide is further fucosylated using a second fucosyltransferase. Such a second fucosyltransferase includes an amino acid sequence substantially identical to the sequence shown in Fig. 6B (SEQ ID NO: 4). Preferably, the fucosylated polypeptide is an AGP-antibody fusion protein or is an antibody (e.g., IgG or IgM).

In related aspects, the invention features a substantially pure polypeptide of the invention which is fucosylated in vivo or in vitro and which is capable of protecting a mammal against an adverse immune reaction. In general, such an adverse immune reaction is septic shock or is septicemia.

In another aspect, the invention features a cell containing at least two recombinant fucosyltransferases, one of the fucosyltransferases being substantially identical to the amino acid sequences shown in Fig 3. (SEQ ID NO: 2) and another of the fucosyltransferases being substantially identical to the amino acid sequence

shown in Fig. 6B (SEQ ID NO: 4). Preferably, such a DNA-containing cell is a prokaryotic cell (e.g., E. coli) or is a eukaryotic cell, for example, a mammalian cell (e.g., the murine cell line 32D c13 or human cell line 293).

In a final aspect, the invention features a method of fucosylating a polypeptide in vitro comprising: (a) providing an α(1,3) fucosyltransferase of the invention; and (b) contacting the polypeptide with the fucosyltransferase under conditions sufficient for fucosylating the polypeptide.

By "polypeptide" is meant any chain of amino acids, regardless of length or post-translational modification (e.g., glycosylation or phosphorylation).

By "substantially identical" is meant a polypeptide exhibiting at least 50%, preferably 70%, more preferably 90%, and most preferably 95% homology to a reference amino acid or is meant a nucleic acid sequence exhibiting at least 85%, preferably 90%, more preferably 95%, and most preferably 97% homology to a reference nucleic acid sequence. For polypeptides, the length of comparison sequences will generally be at least 16 amino acids, preferably at least 20 amino acids, more preferably at least 25 amino acids, and most preferably 35 amino acids. For nucleic acids, the length of comparison sequences will generally be at least 30 nucleotides, preferably at least 60 nucleotides, more preferably at least 75 nucleotides, and most preferably 110 nucleotides.

Sequence identity is typically measured using sequence analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Such software matches similar sequences by assigning degrees of

homology to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

By a "substantially pure polypeptide" is meant a fucosyltransferase polypeptide which has been separated from components which naturally accompany it. Typically, the polypeptide is substantially pure when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, fucosyltransferase polypeptide. A substantially pure fucosyltransferase polypeptide may be obtained, for example, by extraction from a natural source (e.g., a murine cell such as 32D c13); by expression of a recombinant nucleic acid encoding a fucosyltransferase polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A protein is substantially free of naturally associated components when it is separated from those contaminants which accompany it in its natural state. Thus, a protein which is chemically synthesized or produced in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. Accordingly, substantially pure polypeptides include, without limitation, those derived from eukaryotic organisms but synthesized in E. coli or other prokaryotes, or those derived from a eukaryotic cell

which does not normally synthesize such a protein, or those derived from a eukaryotic cell engineered to overexpress such a protein.

By "substantially pure DNA" is meant DNA that is free of the genes which, in the naturally-occurring genome of the organism from which the DNA of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA which is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or which exists as a separate molecule (e.g., a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. It also includes a recombinant DNA which is part of a hybrid gene encoding additional polypeptide sequence.

By "transformed cell" is meant a cell into which (or into an ancestor of which) has been introduced, by means of recombinant DNA techniques, a DNA molecule encoding (as used herein) a fucosyltransferase polypeptide.

By "positioned for expression" is meant that the DNA molecule is positioned adjacent to a DNA sequence which directs transcription and translation of the sequence (i.e., facilitates the production of, e.g., a recombinant fucosyltransferase polypeptide or RNA molecule).

By "promoter" is meant the minimal sequence sufficient to direct transcription. Also included in the invention are those promoter elements which are sufficient to render transcription controllable for cell-type specific, tissue-specific, or inducible expression; such elements may be located in the 5′ or 3′ regions of the native gene.

By "operably linked" is meant that a gene and a regulatory sequence(s) are connected in such a way as to permit gene expression when the appropriate molecules (e.g., transcriptional activator proteins) are bound to the regulatory sequence(s).

By "purified antibody" is meant antibody which is at least 60%, by weight, free from proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably 90%, and most preferably at least 99%, by weight, antibody, e.g., a fucosyltransferase-specific antibody. A purified fucosyltransferase antibody may be obtained, for example, by affinity chromatography using recombinantly-produced fucosyltransferase protein or conserved motif peptides and standard techniques.

By "specifically binds" is meant an antibody which recognizes and binds a fucosyltransferase protein but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample, which naturally includes fucosyltransferase.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Detailed Description

The drawings will first be described.

Drawings

Figure 1 is a flow cytometry profile showing the expression of fucosylated glycans by COS cells transfected with murine myeloid fucosyltransferase. The results are expressed as mean fluorescence intensity in arbitrary units. No expression above background of any carbohydrate epitope except sialyl-Le^x was seen.

Figures 2A-C are panels of autoradiograms showing different nucleic acid blot hybridizations. Panel (A) is an autoradiogram showing a DNA blot hybridization of total genomic DNA from mouse kidney tissue. Murine genomic DNA (15 μg) was digested with the indicated restriction enzymes and subjected to fractionation, transfer, and blot hybridization. Panel (B) is an autoradiogram showing an RNA blot hybridization of total RNA from different cell lines. Total RNA (20 μg) prepared from each of the cell lines shown was denatured, fractionated by gel electrophoresis, transferred to nylon and hybridized. The lineage origins of the cell lines are: YAC-1 (T cell leukemia), EL4 (thymoma), RDM4 (T cell leukemia), CTLL-2 (IL-2 dependent cytotoxic T cell), 32D cl3 (IL-3 dependent granulocyte precursor), WEHI-231 (B cell lymphoma, non-secreting, mouse), WEHI-279 (B cell lymphoma, non-secreting, mouse), Sp2/0 (plasmacytoma), WEHI-3B (myeloid (monocytic) leukemia), P815 (mastocytoma), Ltk (fibroblast), and Balb 3T3 (fibroblast). Panel (C) is an autoradiogram showing an RNA blot hybridization of total RNA from skeletal tissue. Total RNA (20 μg) prepared from skeletal muscle (Sk. muscle) was denatured, fractionated by gel electrophoresis, transferred to nylon and hybridized. Arrows denote the location of ribosomal RNAs.

Figure 3 is the nucleotide (SEQ ID NO: 1) and deduced amino acid sequence (SEQ ID NO: 2) of the murine myeloid-lineage fucosyltransferase cDNA. Two sites for N-linked glycan addition are underlined in the predicted peptide sequence, as is the upstream ATG in the nucleic acid sequence.

Figure 4 is a flow cytometry profile showing the expression of fucosylated glycans by 32D cl3 cells stably transfected with the human myeloid fucosyltransferase. Pooled products of the transfection of 32D cl3 cells with

a human Fuc-TIV myeloid fucosyltransferase expression plasmid bearing a selectable marker were evaluated by indirect immunofluorescence using anti-carbohydrate monoclonal antibodies and flow cytometry. The results are expressed as mean fluorescence intensity in arbitrary units.

Figure 5 is a bar graph of cell adhesion assays (Panels A-B). Panel (A) shows the adhesion to E-selectin or P-selectin IgG fusion proteins of COS cells transfected with either the human Fuc-TIV (FTIV) or the murine 32D cl3 fucosyltransferase (32DFT) cDNAs in the presence or absence of the P-selectin glycoprotein ligand (PSGL). Panel (B) shows adhesion to E-selectin or P-selectin IgM fusion proteins of 32D cl3 cells (32D cl3) or 32D cl3 cells transfected with the human myeloid Fuc-TIV cDNA (FTIV). TLISA, control IgM fusion protein; E, P, E- or P-selectin IgM fusion protein. Binding to P-selectin/IgM fusion protein was not significantly increased by expression of Fuc-TIV. Columns represent average cpm bound of triplicate samples.

Figure 6, panel A is the nucleotide sequence (SEQ ID NO: 3) and, panel B, the deduced amino acid sequence (SEQ ID NO: 4) of the human myeloid-lineage Fuc-TIV cDNA.

Below we describe the isolation of an α(1,3) fucosyltransferase cDNA from a murine myeloid cell line which has a relatively strict substrate requirement for sialylated N-acetyllactosamine, which can account for the presence of the sialyl-Le^x epitope on murine cells, and which is substantially more effective than the previously described myeloid cell fucosyltransferase, Fuc-TIV, in support of E-selectin-mediated COS cell adhesion. We also show that the introduction of the human Fuc-TIV transferase into a murine cell line results in the appearance of a fucosylated glycan pattern similar to that found on human neutrophils and monocytes. Murine

cells expressing human Fuc-TIV show enhanced adhesion to E-selectin fusion proteins, indicating that Fuc-TIV has an important physiological function in the context of human granulocyte extravasation. These examples are presented to illustrate, not limit, the invention.

Cell Lines and Growth Conditions

The following cell lines and growth conditions were used in this study. YAC-1, EL4, RDM4, CTLL2, Sp2/0, WEHI-231, WEHI-279 and P815 cell lines were cultured in IMDM, 10% FCS, 50 mM mercaptoethanol, 50 U/ml penicillin and 50 μg/ml streptomycin. Balb 3T3, Ltk$^-$ and COS-7 m6 cell lines were passaged in DMEM, 10% calf serum (CS) and 25 μg/ml gentamicin sulfate. The IL-3 producing, macrophage-like cell line WEHI-3B was cultured in RPMI-1640, 10% FCS, 50 U/ml penicillin and 50 mg/ml streptomycin. The IL-3 dependent mouse neutrophil progenitor cell line, 32D cl3, was cultured in RPMI-1640, 10% FCS, 10% WEHI 3B-conditioned medium, 50 U/ml penicillin and 50 μg/ml streptomycin (Kreider et al., *Oncogene* 7:135, 1992).

Isolation of cDNA Encoding Murine α(1,3) Fucosyltransferase

To isolate a cDNA clone capable of directing the expression of sialyl-Le^x determinants, an expression library was prepared from mRNA isolated from the murine cell line 32D cl3, which phenotypically resembles a granulocyte precursor and which binds murine E- and P-selectin (Levinovitz et al., *J. Cell Biol.* 121:449, 1993) as follows. A cDNA library in the expression vector CDM8 was prepared from 32D cl3 cells as described by Aruffo et al. (*Proc. Natl. Acad. Sci. USA* 84:8573, 1987) using the introduction of a Mung bean exonuclease treatment followed by T4 DNA polymerase prior to the addition BstX1 adaptors. Following the second strand synthesis, the

cDNA pellet was resuspended in 225 μl distilled water, 25 μl 10X Mung bean incubation buffer (500 mM sodium acetate, 300 mM NaCl, 10 mM zinc sulfate, pH 5.0), and 10 U of Mung bean nuclease (New England Biolabs, Beverly, MA.). After a 10 minute incubation period at 37°C, the reaction was stopped by adding 20 μl of 1 M Tris-HCl, pH 8.0 and 3 μl 0.5 M EDTA, pH 8.0. The cDNA was phenol extracted, ethanol precipitated and resuspended in 90 μl distilled H_2O. Following the addition of 10 μl 10x T4 DNA polymerase buffer (330 mM Tris acetate, 660 mM potassium acetate, 100 mM magnesium acetate, 5 mM DTT, 1 mg/ml bovine serum albumin (BSA), pH 8.0), 1 μl of a mixture containing 25 mM each of the deoxynucleoside triphosphates and 1 U of T4 DNA polymerase (Boehringer-Mannheim), the mixture was incubated at 37°C for 30 min. The reaction was stopped by adding 0.5 μl 0.5 M EDTA, phenol extracted and ethanol precipitated. Following ligation of the BstX1 adaptors, unligated adaptors were removed by repeated centrifugal ultrafiltration through a filter with a molecular weight cut off of 100 kD (Amicon, Danvers, MA), followed by velocity sedimentation fractionation.

The ligated cDNA in CDM8 was introduced into electrocompetent MC1061/p3 cells by electroporation in 0.2 cm gap cuvettes (Bio-Rad laboratories, Hercules, CA) at a voltage of 2.5 kV, a capacitance of 25 μF and a parallel resistance of 400 Ohms. Transformed bacteria were plated on 20 dishes, 23 x 23 cm in size (Nunc, Denmark). Bacteria from each dish (≈ 1.25 X 10^5 colonies) were harvested and an aliquot stored frozen at -70°C in 40% glycerol. Plasmid DNA was isolated from each pool using a commercial kit (plasmid midi prep QIAGEN Inc., Chatsworth, CA) according to the manufacturer's recommendations.

The library was then divided into 20 pools of 1.25 x 10^5 cells each and between 200 and 500 ng of plasmid DNA from each of the 20 pools was separately transfected into COS-7 m6 cells at approximately 70% confluence in a 10 cm-dish using the DEAE-dextran method described by Seed et al. (*Proc. Natl. Acad. Sci USA* 84:3365, 1987). The COS cells were stained with the sialyl-Le^x antibody and bacteria from positive pools replated at lower density. Between 48 and 60 hours post-transfection pools bearing cDNAs capable of directing the appearance of the sialyl-Le^x determinant, dishes with positive cells were identified by immunocytochemistry using an anti-sialyl-Le^x antibody (KM93, mouse IgM; Kamiya Biomedical Company, Thousand Oaks, CA), and an avidin-biotin complex protocol employing 9-amino-3-ethylcarbazol as a peroxidase substrate kit (Vector Labs, Burlingame, CA) essentially as described Horst et al. (*Nucleic Acids Res.* 19:4556, 1991; Vector Labs). Bacteria corresponding to positive pools were subsequently replated at lower density on 10 cm dishes. Plasmid DNA from these subpools was transfected into COS cells in 6 cm dishes. The procedure was repeated until a single plasmid was recovered that conferred binding of anti-sialyl-Le^x antibody to transfected COS cells. Bacterial cells from the pool giving rise to the highest number of positive transfectants were plated at lower density on agar plates and DNA prepared from the bacteria was transfected into COS cells, allowing pools of successively less sequence complexity to be obtained until finally a single clonally pure plasmid isolate was shown to be capable of directing the appearance of the sialyl Lewis-X epitope in COS cells. Five out of twenty pools contained five or more positive cells.

Flow Cytometry Analysis

To confirm the expression of the sialyl Lewis-X epitope, we examined the expression of this epitope in COS cells transfected with the 32D c13 fucosyltransferase expression plasmid described above by indirect immunofluorescence using anti-carbohydrate monoclonal antibodies and flow cytometry as follows. Transfected COS cells to be stained for FACS analysis were harvested by detaching the cells from plates 48 to 60 hours post-transfection using 0.5 mM EDTA in phosphate-buffered saline (PBS). Staining of cells for FACS analysis was done by incubating 2 x 10^6 cells on ice for 20 to 30 minutes in 0.5 ml of 3% BSA in PBS with 4 μg/ml of antibody or in 0.5 ml of hybridoma supernatant for 20 to 30 minutes. For this analysis, the following antibodies were used: anti-Le^x antibody (PM81, mouse IgM; Medarex, Inc., West Lebanon, NH), anti-sialyl-Le^x (KM93, mouse IgM), anti-sialyl-Le^a (KM231, mouse IgG1; Kamiya Biomedical Co., Thousand Oaks, CA), anti-Le^a antibody (T174, mouse IgG1; Signet, Dedham, MA), anti-CD65 (VIM-2) antibody (88H7, mouse IgM; AMAC, Westbrook, ME), and the hybridoma secreting a mouse IgG3 antibody against di- and tri- fucosylated Le^x (FHCR-1-2075/FH4; ATCC, Rockland, MD). Following two washes in PBS, the cells were resuspended in 0.5 ml of 3% BSA in PBS containing 2 μg/ml FITC-conjugated anti-mouse IgG or IgM antibody (Organon Teknika Corp., Durham, NC). Washed cells were immediately analyzed by flow cytometry (Coulter Corp., Hialeah, FA) according to standard methods. Flow cytometric analysis of the transfected cells showed that sialyl-Le^x, but neither Le^x, CD65, di/trimeric Le^x (FH4 epitope), Le^a, nor sialyl-Le^a determinants could be detected (Fig. 1).

DNA Blot (Southern) Hybridization Analysis

To determine gene copy number of the 32D c13 fucosyltransferase gene, DNA blot hybridization was performed as follows. Fifteen micrograms of mouse genomic DNA (Adult, male, Balb/c kidney; Clontech Labs, Palo Alto, CA) was digested overnight in a volume of 300 µl with 50 U of BamH1, 90 U of EcoR1, 100 U of HindIII and 100 U of PstI individually. The digests were phenol extracted, ethanol precipitated and separated on a 0.8% agarose gel. The gel was denatured by incubation in 0.5 M NaOH, 1.5 M NaCl, at room temperature for 30 minutes, briefly rinsed in distilled water, and neutralized for 30 minutes in 0.5 M Tris-HCl, pH 7.0, 1.5 M NaCl at room temperature. Following an incubation in 20X SSC for 30 minutes, the DNA was blotted and probed as for the RNA blots described below. The results of this analysis indicated that the fucosyltransferase is encoded by at least a single copy gene (Fig. 2A).

RNA Blot Hybridization Analysis

To study the expression of the 32D c13 fucosyltransferase gene, RNA blot hybridization was performed as follows. Total RNA was isolated from YAC-1, EL4, RD-M4, CTLL2, 32D c13, WEHI 231, WEHI 279, Sp2/0, WEHI 3B, P815, Ltk- and Balb 3T3 cells using a guanidinium-acid phenol protocol (Chomczynski et al., *Analyt. Biochem.* 162:156, 1987). The heart, stomach, small intestine, large intestine, liver, kidneys, spleen, brain and a piece of skeletal muscle was dissected from a male 129 SVJ mouse sacrificed by cervical dislocation. Total RNA was isolated from the tissues as described above after homogenization on ice in guanidinium thiocyanate buffer using a handheld homogenizer (Omni International, Waterbury, CT). Twenty micrograms of total RNA was separated by electrophoresis in a 1.2%

agarose/formaldehyde gel and transferred to nylon membranes (Schleicher & Schuell, Keene, N.H.) using a downward transfer system (Schleicher & Schuell) according to the manufacturer's recommendations. RNA absorbed to the membrane was crosslinked by UV irradiation (1200 μJ) and detected by hybridization with a randomly primed probe using standard conditions (Ausubel et al., *Current Protocols In Molecular Biology*, Wiley Interscience, 1995).

RNA blot analysis showed a pattern of highly tissue-restricted expression of a message of 1.9 kb (Fig. 2 B and C). Among established cell lines high levels of mRNA were found in 32D cl3 and CTLL-2, an IL-2 dependent cytotoxic T cell line, with the myeloid cell line WEHI 3B and the mastocytoma P815 having significant quantities of the mRNA (Fig. 2B). No message was detected in T-cell lines (YAC-1, EL4 and RDM4), in B cell or fibroblast lines, or in any of the tissues sampled (Fig. 2B).

DNA Sequencing

The sequence of the isolated cDNA clone was determined by the dideoxy chain termination method using modified T7 DNA polymerase (U.S. Biochemical Corp., Cleveland, OH) (Sanger et al., *Proc. Natl. Acad. Sci. USA* 74:5463, 1977). Ambiguities found in the sequence due to self-complementary sequences were resolved using 7-deasa-dGTP.

DNA Sequence Analysis and Deduced Protein Sequence

The cDNA insert consists of 1814 nucleotides, terminating just 3′ to a canonical upstream poly(A) sequence motif (Fig. 3). The largest open reading frame begins at a methionine at position 325 which does not meet the sequence requirements for a translational initiation consensus, but is preceded by only one other

candidate ATG, similarly lacking a consensus initiation context and giving rise to a translation product which terminates within a few residues. The predicted polypeptide contains a long amino-terminal hydrophobic region preceded by arginine residues, similar in structure to the transmembrane domain of type II (amino terminally anchored) integral membrane proteins typical of this class of glycosyltransferase. A carboxyl-terminal sequence from peptide residues 245 to 258 consisting largely of aliphatic hydrophobic amino acids, although reminiscent of the membrane spanning domains of type I integral membrane proteins, is probably too short to serve a membrane insertion function. The predicted molecular mass of the encoded protein is 39.4 kilodaltons, with the presence of two N-linked glycan addition sites at residues 81 and 291 suggesting that the mature protein may be larger.

Comparison of protein sequences showed that the 32D cl3 fucosyltransferase shares identity at 47% of residues with the human myeloid fucosyltransferase and at 80% of residues with the human type VII fucosyltransferase. Given that the N-acetyllactosamine fucosyltransferases constitute a large and growing family, it is likely that another unidentified human isolate may prove more closely related to the present 32D c13 fucosyltransferase enzyme. Such a gene is isolated using the techniques described herein.

Expression of Human Fucosyltransferase in Murine Cells

The cDNA insert of a previously isolated expression clone encoding human myeloid α(1,3) fucosyltransferase (Fuc-TIV; Fig. 6A, SEQ ID NO: 3) was excised from the πH3m vector (Aruffo et al. *Proc. Natl. Acad. Sci. USA* 84:8573, 1987) with HindIII and HpaI and subcloned into the polylinker of a bidirectional vector

bearing the Spleen-focus forming virus (Sffv) LTR upstream of a polylinker, a splice donor and acceptor site, and the bidirectional poly(A) addition signal from SV40; opposite in orientation to this transcription unit, and utilizing the poly(A) signals from the opposite direction was a second transcription unit consisting of the HSV TK promoter followed by the coding sequences for puromycin acetyltransferase. The Sffv Fuc-TIV plasmid was linearized by digestion with Avr2, phenol extracted, ethanol precipitated, and electroporated into the 32D cl3 cell line as follows. The cells (8×10^7) were resuspended in 0.8 ml RPMI-1640, 10% FBS, 10% WEHI-3B conditioned medium, and transferred together with 40 μg of linearized plasmid DNA to a 0.4 cm-gap electroporation cuvette (Bio-Rad, Hercules, CA) on ice. A single pulse was delivered at a voltage of 250V and a capacitance of 500 μF. After electroporation, the cuvette was put back on ice for 10 minutes before the cells were transferred to a flask containing 50 ml of medium. Puromycin was added to the medium the following day at a concentration of 0.5 μg/ml. After approximately 2 weeks, with media changes every second to third day, the cells were checked for expression of the sialyl-Le^x epitope.

Transfection of the human myeloid cell-specific fucosyltransferase cDNA (Fuc-TIV; Fig. 6A, SEQ ID NO: 3) into a murine granulocytic cell line resulted in the appearance of glycan epitope pattern similar to that of human cells (Fig. 4). Specifically, the levels of expression of Le^x (CD15) and NeuNAcα3Galβ4GlcNAcβ3Galβ4(Fucα3)GlcNAc (CD65) epitopes were markedly increased, and substantial levels of multiply fucosylated poly N-acetyllactosamine epitopes were also detected. Together these findings support the notion that internally fucosylated poly N-

acetyllactosamine chains are present in the transfectant but not parental cells.

Fusion Proteins

The construction of DNA sequences coding for fusions between E- and P-selectin extracellular domains (for P-selectin only 2 of the complement regulatory domains were included) and the Fc part (hinge, CH2 and CH3) of human genomic IgG1 was performed as previously described (Walz et al., *Science* 250:1132, 1990; Aruffo et al., *Cell* 61:1303, 1990). The cDNA sequences for E- and P-selectin extracellular domains were fused to the genomic sequence of human IgM Fc (CH2, CH3 and CH4) by transferring the selectin sequences from an IgG fusion vector to an IgM fusion vector created in this laboratory (Zettlmeissl et al., *DNA Cell Biol.* 9:347, 1990). The PSGL-1 cDNA coding sequence was obtained by PCR from an HL-60 cDNA library and confirmed by DNA sequencing. The coding segment for the mature extracellular, transmembrane and intracellular domain was inserted in an expression vector based on CDM8 which lacks the polyoma virus origin of replication and contains the leader sequence for the CD5 antigen positioned just upstream of the coding region for an influenza hemagglutinin peptide epitope tag.

COS cell supernatants containing soluble E- and P-selectin/IgG and IgM fusion proteins were produced as previously described (Walz et al., *Science* 250:1132, 1990; Aruffo et al., *Cell* 61:1303, 1990). The concentration of fusion protein in the tissue culture supernatants was determined by a 96-well ELISA assay, in which the fusion proteins were captured with an affinity purified, polyclonal anti-human IgG Fc or anti-human IgM (μ chain specific) antibody (Organon Teknika, Durham, NC). Captured fusion proteins were detected with a

peroxidase-conjugated, affinity purified, polyclonal anti-human IgG Fc or anti-human IgM (μ chain specific) antibody (Organon Teknika) using O-phenylenediamine dihydrochloride as substrate (Sigma). The ELISA was calibrated using purified human IgG or IgM (Sigma).

Adhesion Assays

Adhesion assays were performed in 96-well ELISA plates (Becton-Dickinson, Oxnard, CA) as follows. The wells were incubated with 100 μl of 20 μg/ml anti-human IgG Fc or anti-human IgM (heavy chain specific) in PBS for 2 hrs in a humid chamber at room temperature. After washing the plate twice with PBS, additional protein-binding sites were blocked by an overnight incubation with 200 μl 3% BSA in PBS. The plate was washed with PBS four times and incubated with 200 μl of fusion protein supernatants for 2 hrs. Following three PBS washes and one wash in 0.2% BSA, 0.15 M NaCl, 3 mM $CaCl_2$, 2 x 10^5 cells/well in 200 μl 0.2% BSA, 0.15 M NaCl, 3 mM $CaCl_2$ were added and left to bind for 15 minutes in room temperature having the plate on a rotary platform (60 rpm). The plate was washed three times by carefully dropping in 200 μl 0.15 M NaCl, 3 mM $CaCl_2$ in the wells and then carefully inverting the plate on a pile of paper towels in order to gently pour out the liquid. Transfected cells used for the assay were lifted off the dish with 0.5 mM EDTA in PBS 48 to 60 hrs after transfection and loaded with 100 μl $Na_2{}^{51}CrO_4$ (1 mCi/ml; DuPont, Boston, MA) in 0.9% NaCl plus 100 ml medium at 37°C for 1 hr. Loaded cells were washed twice in PBS and resuspended in 0.2% BSA, 0.15 M NaCl, 3 mM $CaCl_2$. Adherent cells were lysed by the addition of 200 μl 2% SDS and counted in a gamma ray spectrometer.

The results of these experiments showed that COS cells transfected with the 32D cl3 fucosyltransferase

cDNA had significantly greater binding to immobilized E- and P-selectin than did the human Fuc-TIV (myeloid) enzyme; the binding to P-selectin was observed only when the COS cells were cotransfected with a cDNA expression vector encoding PSGL-1 (Fig. 5A).

The 32D cl3 cell line itself binds both human E- and P-selectin/IgM fusion proteins (Fig. 5B). IgM fusion proteins were used in these experiments to avoid the possible contribution of Fc receptor binding. To evaluate the functional consequences of a human-like fucosylated glycan spectrum, 32D cl3 cells stably expressing the human Fuc-TIV (myeloid) enzyme (Fig. 6B) were evaluated in the selectin adhesion assay. The transfectants showed an approximately 10-fold higher binding density to human E-selectin relative to untransfected cells, whereas binding to P-selectin was not significantly affected (Fig. 5).

Isolation of Fucosyltransferase cDNA and Genomic DNA

Cloning and isolation of fucosyltransferase cDNA according to the invention is carried out according to the methods described herein. Cloning of genomic DNA is performed according to well known methods.

Based on our discovery of a novel myeloid-expressed fucosyltransferase, the isolation of additional mammalian fucosyltransferases, including human fucosyltransferases, is made possible using standard techniques. In particular, using all or a portion of the amino acid sequence of a fucosyltransferase of the invention, one may readily design fucosyltransferase oligonucleotide probes, including fucosyltransferase degenerate oligonucleotide probes (i.e., a mixture of all possible coding sequences for a given amino acid sequence). These oligonucleotides may be based upon the sequence of either strand of the DNA comprising the

motif. General methods for designing and preparing such probes are provided, for example, in Ausubel et al., supra, and *Guide to Molecular Cloning Techniques*, 1987, S. L. Berger and A. R. Kimmel, eds., Academic Press, New York. These oligonucleotides are useful for fucosyltransferase gene isolation, either through their use as probes capable of hybridizing to fucosyltransferase complementary sequences or as primers for various polymerase chain reaction (PCR) cloning strategies. In one particular example, isolation of other fucosyltransferase genes is performed by PCR amplification techniques well known to those skilled in the art of molecular biology using oligonucleotide primers designed to amplify only sequences flanked by the oligonucleotides in genes having sequence identity to fucosyltransferase of the invention. The primers are optionally designed to allow cloning of the amplified product into a suitable vector.

Hybridization techniques and procedures are well known to those skilled in the art and are described, for example, in Ausubel et al., supra, and *Guide to Molecular Cloning Techniques*, supra. If desired, a combination of different oligonucleotide probes may be used for the screening of the recombinant DNA library. The oligonucleotides are labelled with ^{32}P using methods known in the art, and the detectably-labelled oligonucleotides are used to probe filter replicas from a recombinant DNA library. Recombinant DNA libraries may be prepared according to methods well known in the art, for example, as described in Ausubel et al., supra, or may be obtained from commercial sources.

For detection or isolation of closely related fucosyltransferases, high stringency conditions may be used; such conditions include hybridization at about 42°C and about 50% formamide; a first wash at about 65°C,

about 2X SSC, and 1% SDS; followed by a second wash at about 65°C and about 0.1% SDS, 1X SSC. Lower stringency conditions for detecting fucosyltransferase genes having about 85% sequence identity to the fucosyltransferase gene described herein include, for example, hybridization at about 42°C in the absence of formamide; a first wash at about 42°C, about 6X SSC, and about 1% SDS; and a second wash at about 50°C, about 6X SSC, and about 1% SDS.

As discussed above, fucosyltransferase oligonucleotides may also be used as primers in PCR cloning strategies. Such PCR methods are well known in the art and described, for example, in *PCR Technology*, H.A. Erlich, ed., Stockton Press, London, 1989; *PCR Protocols: A Guide to Methods and Applications*, M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds., Academic Press, Inc., New York, 1990; and Ausubel et al., supra. If desired, fucosyltransferases may be isolated using the PCR "RACE" technique, or Rapid Amplification of cDNA Ends (see, e.g., Innis et al., supra). By this method, oligonucleotide primers based on a fucosyltransferase conserved domain are oriented in the 3′ and 5′ directions and are used to generate overlapping PCR fragments. These overlapping 3′- and 5′-end RACE products are combined to produce an intact full-length cDNA. This method is described in Innis et al., supra; and Frohman et al., *Proc. Natl. Acad. Sci. USA* 85:8998, 1988.

Fucosyltransferase Polypeptide Expression

Fucosyltransferases according to the invention may be expressed or produced by transformation of a suitable host cell with all or part of a fucosyltransferase-encoding cDNA fragment (e.g., the cDNA described herein)

in a suitable expression vehicle (e.g., those described herein).

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. For example, a fucosyltransferase may be produced in a prokaryotic host (e.g., E. coli) or in a eukaryotic host (e.g., Saccharomyces cerevisiae or mammalian cells, e.g., 32D c13, human cell line 293, COS 1, NIH 3T3, and JEG3 cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, MD; also, see, e.g., Ausubel et al., supra). The method of transformation and the choice of expression vehicle will depend on the host system selected. Transformation methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in *Cloning Vectors*: A Laboratory Manual (P.H. Pouwels et al., 1985, Supp. 1987).

One preferred expression system is the mouse 3T3 fibroblast host cell transfected with a pMAMneo expression vector (Clontech, Palo Alto, CA). pMAMneo provides: an RSV-LTR enhancer linked to a dexamethasone-inducible MMTV-LTR promotor, an SV40 origin of replication which allows replication in mammalian systems, a selectable neomycin gene, and SV40 splicing and polyadenylation sites. DNA encoding a fucosyltransferase polypeptide is inserted into the pMAMneo vector in an orientation designed to allow expression. The recombinant fucosyltransferase is isolated as described below. Other preferable host cells which may be used in conjunction with the pMAMneo expression vehicle include COS cells and CHO cells (ATCC Accession Nos. CRL 1650 and CCL 61, respectively).

More preferably, fucosyltransferase of the invention is expressed or produced by a stably-

transfected mammalian cell line (e.g., 32D c13, or human cell line 293) using the methods and vectors described herein.

In addition, a number of other vectors suitable for stable transfection of mammalian cells are available to the public, e.g., see Pouwels et al. (supra); methods for constructing such cell lines are also publicly available, e.g., in Ausubel et al. (supra). In one example, cDNA encoding the fucosyltransferase polypeptide is cloned into an expression vector which includes the dihydrofolate reductase (DHFR) gene. Integration of the plasmid and, therefore, the fucosyltransferase-encoding gene into the host cell chromosome is selected for by inclusion of 0.01-300 μM methotrexate in the cell culture medium (as described in Ausubel et al., supra). This dominant selection can be accomplished in most cell types. Recombinant protein expression can be increased by DHFR-mediated amplification of the transfected gene. Methods for selecting cell lines bearing gene amplifications are described in Ausubel et al. (supra); such methods generally involve extended culture in medium containing gradually increasing levels of methotrexate. DHFR-containing expression vectors commonly used for this purpose include pCVSEII-DHRF and pAdD26SV(A) (described in Ausubel et al., supra). Any of the host cells described above or, preferably, a DHFR-deficient CHO cell line (e.g., CHO DHFR$^-$cells, ATCC Accession No. CRL 9096) are among the host cells preferred for DHFR selection of a stably-transfected cell line or DHFR-mediated gene amplification.

Once the recombinant fucosyltransferase polypeptide is expressed, it is isolated, e.g., using affinity chromatography. In one example, an anti-fucosyltransferase antibody (e.g., produced as described below) may be attached to a column and used to isolate

the polypeptide. Lysis and fractionation of fucosyltransferase-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra). Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, *Laboratory Techniques In Biochemistry And Molecular Biology*, eds., Work and Burdon, Elsevier, 1980).

These general techniques of polypeptide expression and purification can also be used to produce and isolate useful fucosyltransferase fragments or analogs (described below).

Identification of Molecules Which Modulate Fucosyltransferase Expression

Isolation of the fucosyltransferase gene also facilitates the identification of molecules which increase or decrease fucosyltransferase expression, and which are therefore useful as therapeutics, e.g., for treatment of inflammation. According to one approach, candidate molecules (e.g., peptide or non-peptide molecules found, e.g., in a cell extract, mammalian serum, or growth medium on which mammalian cells have been cultured, or oligonucleotides) are added at varying concentrations to the culture medium of cells which express fucosyltransferase mRNA (e.g., 32D c13). Fucosyltransferase expression is then measured by standard Northern blot analysis (Ausubel et al., supra) using fucosyltransferase cDNA as a hybridization probe. The level of fucosyltransferase expression in the presence of the candidate molecule is compared to the level measured for the same cells in the same culture medium but in the absence of the candidate molecule. A molecule which promotes an increase or decrease in

fucosyltransferase expression is considered useful in the invention.

Anti-Fucosyltransferase Antibodies

Fucosyltransferases described herein (or immunogenic fragments or analogues) may be used to raise antibodies useful in the invention; such polypeptides may be produced by recombinant or peptide synthetic techniques (see, e.g., *Solid Phase Peptide Synthesis*, supra; Ausubel et al., supra). The peptides may be coupled to a carrier protein, such as KLH as described in Ausubel et al, supra. The KLH-peptide is mixed with Freund's adjuvant and injected into guinea pigs, rats, or preferably rabbits. Antibodies may be purified by peptide antigen affinity chromatography.

Monoclonal antibodies may be prepared using the fucosyltransferase polypeptides described above and standard hybridoma technology (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., *In Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, NY, 1981; Ausubel et al., supra).

Once produced, polyclonal or monoclonal antibodies are tested for specific fucosyltransferase recognition by Western blot or immunoprecipitation analysis (by the methods described in Ausubel et al., supra). Antibodies which specifically recognize fucosyltransferase are considered to be useful in the invention; such antibodies may be used, e.g., in an immunoassay to monitor the level of fucosyltransferase produced by a mammal.

Fucosylation and Production of Sialyl-Le^x Determinants

The invention features genes, enzymes, and methods for fucosylating virtually any protein bearing one or more glycan addition sites, e.g., an N-linked glycan

addition site. By "N-linked" is meant bonded to the amide nitrogen of an asparagine residue of a protein. For example, it has been discovered that antibodies (as described in Seed et al., USSN 08/472,888, entitled "AGP-Antibody Fusion Proteins and Related Molecules and Methods," filed June 7, 1995) bearing one or more genetically-engineered carbohydrate determinants mask the CH2 portion of the immunoglobulin molecule and thus inhibit complement fixation and F_c receptor binding. Such antibodies are useful for disrupting undesirable interactions between cells or proteins, or, generally, for disrupting an interaction between any two molecules, one of which bears a determinant specifically recognized by an antibody. Because the carbohydrate moieties block the immunoglobulin domain which triggers complement fixation and F_c receptor binding, such antibodies do not elicit the undesirable side effects (i.e., those resulting from complement fixation and F_c receptor binding) frequently associated with antibody-based therapies. Preferably, the carbohydrate groups serve not only to inhibit undesirable complement fixation and F_c receptor binding, but also perform the function of competitively inhibiting a carbohydrate ligand-cell adhesion protein interaction. Where the carbohydrate groups perform this function, the antibody generally does not serve any function arising from its specificity, but serves only as a carrier for the carbohydrate groups. There is described herein such a molecule, in which the carbohydrate side chain includes the sialyl-Le^x determinant.

Sialyl-Le^x normally acts to facilitate interaction between cells which bear it (e.g., neutrophils) and cells which bear the protein, ELAM-1 or E-selectin (e.g., endothelial cells, e.g., those lining the blood vessel walls). Disrupting this interaction has therapeutic

applications, for example, in minimizing inflammation, such as that which occurs following tissue injury, e.g., myocardial infarction, which is characteristic of diseases such as psoriasis or rheumatoid arthritis, or for preventing or inhibiting septicemia or septic shock which is induced by a microbial- or host-mediated immune reaction.

According to one example, the gene encoding a protein bearing a sialyl-Le^x determinant, e.g., an IgG1 antibody or an α_1-AGP-antibody fusion, is inserted into a vector designed to express the protein in a eukaryotic cell (see, e.g., those vectors described in Gillies et al., U.S. Patent No. 4,663,281, hereby incorporated by reference). The eukaryotic host cell is preferably a mammalian cell (e.g., 32D c13, or human cell line 293, or a CHO, or lec11 cell), and the expression vector containing the sialyl-Le^x-encoding sequence is introduced into the host cell by transient or stable transfection using standard techniques. Such host cells are also transfected (transiently or stably) with a vector capable of expressing an α(1,3)fucosyltransferase of the invention (i.e., an enzyme capable of attaching one or more sialyl-Le^x groups to the protein molecule at sialyl-Le^x consensus glycosylation sites (N-X-T/S)). The α(1,3)fucosyltransferase gene described herein or a combination of the α(1,3)fucosyltransferase gene described herein and the Fuc-TIV gene may be expressed from a vector distinct from that encoding the protein containing sialyl-Le^x addition sites, or, if desired, the genes may be carried on, and expressed from, a common vector. Mammalian cells are particularly useful hosts for the synthesis of sialyl-Le^x modified proteins because they provide all required precursors for sialyl-Le^x production.

Proteins (e.g., antibodies, AGP, or AGP-antibody fusions) which are fucosylated according to the methods of the invention have important therapeutic and diagnostic uses. Previous work has demonstrated that large amounts of antibody fusion proteins may be generated and secreted transiently from transfected mammalian cells (for example, COS cells). In general, to produce an antibody fusion protein, cDNA encoding a domain of interest is fused in-frame, for example, to human IgG domains (for example, constant domains) by standard techniques, and the fusion protein is expressed. The antibody portion of the molecule facilitates fusion protein purification and also prolongs the plasma half-life of otherwise short-lived polypeptides or polypeptide domains. Recombinant plasmids expressing α_1-AGP-IgG1 fusion proteins (e.g., α_1-AGP-Hinge-CH2-CH3 and α_1-AGP-CH2-CH3) are disclosed in Seed et al., USSN 08/472,888, entitled "AGP-Antibody Fusion Proteins and Related Molecules and Methods," filed June 7, 1995.

Host cells expressing the α(1,3)fucosyltransferase of the invention or a combination of any α(1,3)fucosyltransferase of the invention and Fuc-TIV (e.g., SEQ ID NO: 4) along with a protein which is to be fucosylated, e.g., IgG1 or an AGP-antibody fusion, are grown by standard methods and the fucosylated protein purified by standard techniques (for example, for an antibody or antibody fusion protein, using a Protein A column).

Alternatively, any protein, e.g., IgG1 or an AGP-antibody fusion, bearing sialyl-Le^x addition sites may be fucosylated <u>in vitro</u> using any of the enzymes or any combination of enzymes described herein according to standard methods known in the art. Again, such <u>in vitro</u> fucosylated proteins can be purified using any standard technique of isolation and purification.

Fucosyltransferase Kits

Kits for carrying out any of the methods disclosed herein are also included in the invention. Such kits generally include a gene encoding the α(1,3)fucosyltransferase of the invention (for example, a fucosyltransferase gene encoding a polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3; SEQ ID NO: 2). Such a kit may also include a gene encoding Fuc-TIV (for example, a human Fuc-TIV polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B; SEQ ID NO: 4) and/or a cell useful for expressing one or more fucosyltransferase genes. Alternatively, a kit according to the invention may include a transformed cell harboring an α(1,3) fucosyltransferase gene described herein, optionally in combination with a Fuc-TIV-encoding gene. Preferably, such fucosyltransferases are expressed in the 32D c13 cell line or human cell line 293. For identifying modulators of the fucosyltransferases described herein, a kit may include a fragment of an α(1,3)fucosyltransferase nucleic acid sequence useful for hybridization purposes, and may also include means for detecting and quantitating α(1,3)fucosyltransferase RNA hybridization.

Other kits according to the invention include substantially pure α(1,3)fucosyltransferase polypeptide (for example, a fucosyltransferase polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3; SEQ ID NO: 2). Such a kit may also include substantially pure Fuc-TIV polypeptide (for example, a fucosyltransferase polypeptide including an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B; SEQ ID NO: 4). Such fucosyltransferase kits are useful for fucosylating a molecule <u>in vitro</u>.

Other Embodiments

Polypeptides according to the invention include the entire murine fucosyltransferase sequence (as shown in Fig. 3; SEQ ID NO: 2) as well as any analog or fragment of the murine fucosyltransferase.

Polypeptides of the invention also include all mRNA processing variants (e.g., all products of alternative splicing or differential promoter utilization) as well as analogous fucosyltransferases from other mammals, including humans.

Specific fucosyltransferase fragments or analogues of interest include full-length or partial (see below) proteins including an amino acid sequence which differs only by conservative amino acid substitutions, for example, substitution of one amino acid for another of the same class (e.g., valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitutions, deletions, or insertions located at positions of the amino acid sequence which do not destroy enzymatic activity (as assayed above or according to any other standard method). Analogs also include fucosyltransferase polypeptides which are modified for the purpose of increasing peptide stability; such analogs may contain, e.g., one or more desaturated peptide bonds or D-amino acids in the peptide sequence or the peptide may be formulated as a cyclized peptide molecule.

Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The General Hospital Corporation

(ii) TITLE OF INVENTION: FUCOSYLTRANSFERASE GENES AND USES THEREOF

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Fish & Richardson P.C.
(B) STREET: 225 Franklin Street
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110-2804

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/483,151
(B) FILING DATE: 07-JUN-1995
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Lech, Karen F.
(B) REGISTRATION NUMBER: 35,238
(C) REFERENCE/DOCKET NUMBER: 00786/278WO1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 617/542-5070
(B) TELEFAX: 617/542-8906
(C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1814 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GTAGCCAAGG TTCCTCTCCA TCTCACCAGA GCCTGCTGGA GGGGAATCAA ACAAGCCTGG 60

ACCTGAGGCT GGGACTAGCT TTCCTGTTTC TGGAGTGGAT GCCAACCCCC TGCCCACCAG 120

CCTGCCTGTC CACGCCAGGG ACACACAGAC TCCTTCCCTT TCCAGACTGG AAAGCCCCCT 180

CCTGGGAGAG CAGGAAGGAA GCAACCTGCA ACTCTTCCAG CCCTGGACCT TGGGCTGAAC 240

CTACAGTTCA AGGGTGCCTC TGTTGGAGAG GCTGCTGTGA TTTGAAAATC TTCTTTCCTT 300

GGTGACAATT CCAGAAGGCT CCAGATGAAT TGTATTGGGT ACCACCCCAC CAGGAGGCTG 360

CGGGCCTGGG GCGGCCTAGC TGGAGGAGCA ACATTCATGG TAATTTGGTT TTTCTGGCTG 420

TGGGGATCAG CTCCTGGAAG TGCCCCTGTG CCTCAGTCCA CACTCACCAT CCTTATCTGG 480

CACTGGCCTT TCACCAACCG GCCGCCAGAG CTACCTGGTG ACACCTGCAC TCGCTATGGC 540

ATGGCCAGCT GCCGTCTGAG TGCTAACCGG AGCCTGCTAG CCAGTGCTGA TGCTGTGGTC 600

TTCCACCACC GTGAGCTGCA AACCCGGCAA TCTCTCCTAC CCCTGGACCA GAGGCCACAC 660

GGACAGCCTT GGGTCTGGGC CTCCATGGAA TCGCCCAGTA ATACCCATGG TCTCCATCGC 720

TTCCGGGGCA TCTTCAACTG GGTGCTGAGC TATCGGCGTG ATTCAGATAT CTTTGTACCC 780

TACGGTCGCT TGGAGCCTCT CTCTGGGCCC ACATCCCCAC TACCGGCCAA AAGCAGGATG 840

GCTGCCTGGG TGATCAGCAA TTTCCAGGAG CGGCAGCAGC GTGCAAAGCT GTACCGGCAG 900

CTGGCCCCTC ATCTGCAGGT GGATGTGTTC GGTCGCGCCA GCGGACGGCC CCTATGCGCT 960

AATTGTCTGC TGCCCACTTT GGCCCGGTAC CGCTTCTACC TGGCCTTTGA GAACTCACAG 1020

CATCGGGACT ACATCACTGA GAAGTTCTGG CGCAATGCCC TGGCGGCTGG TGCTGTACCC 1080

GTGGCGCTGG GACCTCCTCG GGCCACCTAC GAGGCTTTTG TGCCACCAGA TGCCTTTGTA 1140

CACGTGGACG ACTTCAGCTC TGCCCGTGAA CTGGCTGTCT TCCTCGTCAG CATGAATGAG 1200

AGTCGTTATC GTGGCTTCTT TGCTTGGCGA GACCGGCTCC GTGTGCGGCT CCTGGGTGAC 1260

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TATGAAGACC TTGAAAGCTG GTTCCAGGCT TGAACTCCTG CTGCTGGGAG AGGCTGGATG 1380

GGTGGGAGAC TGATGTTGAA ACCAAAGAGC TGGGCATCCA GGCTTTTGGT CACCATGGCA 1440

CTACCCCAAG GCTTTTCCTG TTCAGTGAGC AGGAATTCAG GATATAAGGA GAAAACTGGG 1500

CTGAGATGCC TGGTGGGCTT TAGAGTAGGG GCCCAGGATA AGAGACAATG AATTAATGAG 1560

GAGCATATGG GGAAGGTGGC TGAGGGTCCC TGACTTACCT TGACCCATGG CTGAAGGCTC 1620

CATGCCCATG GCTGGAGCTG GGACCCTACA CTTCTATAGT CAAGGTGCTT AGCCTCAAGG 1680

TTGCAGATGC ACCCTCTAGT ACTCTGGGTG CAGACTGTAC ACTGGGCGCA GGGGGTTGTG 1740

GAAGGACAGT GCAGATGATT CTGGGCTTTT GACACCACAG TTCCCCCAGG GAAAGAGGCA 1800

CTACTAATAA AAAC 1814

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 342 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Met Asn Cys Ile Gly Tyr His Pro Thr Arg Arg Leu Arg Ala Trp Gly
 1               5                  10                  15

Gly Leu Ala Gly Gly Ala Thr Phe Met Val Ile Trp Phe Phe Trp Leu
            20                  25                  30

Trp Gly Ser Ala Pro Gly Ser Ala Pro Val Pro Gln Ser Thr Leu Thr
        35                  40                  45

Ile Leu Ile Trp His Trp Pro Phe Thr Asn Arg Pro Pro Glu Leu Pro
    50                  55                  60

Gly Asp Thr Cys Thr Arg Tyr Gly Met Ala Ser Cys Arg Leu Ser Ala
65                  70                  75                  80

Asn Arg Ser Leu Leu Ala Ser Ala Asp Ala Val Val Phe His His Arg
                85                  90                  95

Glu Leu Gln Thr Arg Gln Ser Leu Leu Pro Leu Asp Gln Arg Pro His
            100                 105                 110

Gly Gln Pro Trp Val Trp Ala Ser Met Glu Ser Pro Ser Asn Thr His
        115                 120                 125

Gly Leu His Arg Phe Arg Gly Ile Phe Asn Trp Val Leu Ser Tyr Arg
    130                 135                 140

Arg Asp Ser Asp Ile Phe Val Pro Tyr Gly Arg Leu Glu Pro Leu Ser
145                 150                 155                 160

Gly Pro Thr Ser Pro Leu Pro Ala Lys Ser Arg Met Ala Ala Trp Val
                165                 170                 175

Ile Ser Asn Phe Gln Glu Arg Gln Gln Arg Ala Lys Leu Tyr Arg Gln
            180                 185                 190

Leu Ala Pro His Leu Gln Val Asp Val Phe Gly Arg Ala Ser Gly Arg
        195                 200                 205

Pro Leu Cys Ala Asn Cys Leu Leu Pro Thr Leu Ala Arg Tyr Arg Phe
    210                 215                 220

Tyr Leu Ala Phe Glu Asn Ser Gln His Arg Asp Tyr Ile Thr Glu Lys
225                 230                 235                 240

Phe Trp Arg Asn Ala Leu Ala Ala Gly Ala Val Pro Val Ala Leu Gly
                245                 250                 255

Pro Pro Arg Ala Thr Tyr Glu Ala Phe Val Pro Pro Asp Ala Phe Val
            260                 265                 270

His Val Asp Asp Phe Ser Ser Ala Arg Glu Leu Ala Val Phe Leu Val
        275                 280                 285

Ser Met Asn Glu Ser Arg Tyr Arg Gly Phe Phe Ala Trp Arg Asp Arg
    290                 295                 300

Leu Arg Val Arg Leu Leu Gly Asp Trp Arg Glu Arg Phe Cys Thr Ile
305                 310                 315                 320
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Cys Ala Arg Tyr Pro Tyr Leu Pro Arg Ser Gln Val Tyr Glu Asp Leu
325 330 335

Glu Ser Trp Phe Gln Ala
340

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2134 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGCACGCTGC CTGTTCGCGC CATGGGGGCA CCGTGGGGCT CGCCGACGGC GGCGGCGGGC 60

GGGCGGCGCG GGTGGCGCCG AGGCCGGGGG CTGCCATGGA CCGTCTGTGT GCTGGCGGCC 120

GCCGGCTTGA CGTGTACGGC GCTGATCACC TACGCTTGCT GGGGGCAGCT GCCGCCGCTG 180

CCTGGGCGTC GCCAACCCCG TCGCGACCGG TGGGCGTGCT GCTGTGGTGG GAGCCCTTCG 240

GGGGCGCGAT CAGCGCCCCG AGGCCGCCCC CTGACTGCCG GCTGCGCTTC AACATCAGCG 300

GCTGCCGCCT GCTCACCGAC ACGCGCGTCC TACGGAGAGG CTCAGGCCGT GCTTTTCCAC 360

CACCGCGACC TCGTGAAGGG GCCCCCCGAC TGGCCCCCGC CCTGGGGCAT CCAGGCGCAC 420

ACTGCCGAGG AGGTGGATCT GCGCGTGTTG GACTACGAGG AGGCAGCGGC GGCGGCAGAA 480

GCCCTGGCGA CCTCCAGCCC CAGGCCCCGG GCCAAGCGCT GGGTTTGGAT GAACTTCGAG 540

TCGCCCTCGC ACTCCCCGGG GCTGCGAAGC CTGGCAAGTA ACCTCTTCAA CTGGACGCTC 600

TCCTACCGGG CGGACTCGGA CGTCTTTGTG CCTTATGGCT ACCTCTACCC CAGAAGCCAC 660

CCCGGCGACC CGCCCTCAGG CCTGGCCCCG CCACTGTCCA GGAAACAGGG GCTGGTGGCA 720

TGGGTGGTGA GCCACTGGGA CGAGCGCCAG GCCCGGGTCC GCTACTACCA CCAACTGAGC 780

CAACATGTGA CCGTGGACGT GTTCGGCCGG GGCGGGCCGG GGCAGCCGGT GCCCGAAATT 840

GGGCTCCTGC ACACAGTGGC CCGCTACAAG TTCTACCTGG CTTTCGAGAA CTCGCAGCAC 900

CTGGATTATA TCACCGAGAA GCTCTGGCGC AACGCGTTGC TCGCTGGGGC GGTGCCGGTG 960

GTGCTGGGCC CAGACCGTGC CAACTACGAG CGCTTTGTGC CCCGCGGCGC CTTCATCCAC 1020

GTGGACGACT TCCCAAGTGC CTCCTCCCTG GCCTCGTACC TGCTTTTCCT CGACCGCAAC 1080

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GCAATTGGGC TCCCTTTGCT GCTGATGGGC ATCATTGTTT AGGGGTGAAG GAGGGGGTTC 1500

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GTAGAAGGAG CCTTGTTGGT GGAGAGTGGA AGGACTGTGG CTGCAGGTGG GACTTTGTTG 1740

TTTGGATTCC TCACAGCCTT GGCTCCTGAG AAAGGTGAGG AGGGCAGTCC AAGAGGGGCC 1800

GCTGACTTCT TTCACAAGTA CTATCTGTTC CCCTGTCCTG TGAATGGAAG CAAAGTGCTG 1860

GATTGTCCTT GGAGGAAACT TAAGATGAAT ACATGCGTGT ACCTCACTTT ACATAAGAAA 1920

TGTATTCCTG AAAAGCTGCA TTTAAATCAA GTCCCAAATT CATTGACTTA GGGGAGTTCA 1980

GTATTTAATG AAACCCTATG GAGAATTTAT CCCTTTACAA TGTGAATAGT CATCTCCTAA 2040

TTTGTTTCTT CTGTCTTTAT GTTTTTCTAT AACCTGGATT TTTTAAATCA TATTAAAATT 2100

ACAGATGTGA AAATAAAAAA AAAAAAAAAA AAAA 2134

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 405 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: not relevant
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Gly Ala Pro Trp Gly Ser Pro Thr Ala Ala Ala Gly Gly Arg Arg
1 5 10 15

Gly Trp Arg Arg Gly Arg Gly Leu Pro Trp Thr Val Cys Val Leu Ala
20 25 30

Ala Ala Gly Leu Thr Cys Thr Ala Leu Ile Thr Tyr Ala Cys Trp Gly
35 40 45

Gln Leu Pro Pro Leu Pro Trp Ala Ser Pro Thr Pro Ser Arg Pro Val
50 55 60

Gly Val Leu Leu Trp Trp Glu Pro Phe Gly Gly Ala Ile Ser Ala Pro
65 70 75 80

Arg Pro Pro Pro Asp Cys Arg Leu Arg Phe Asn Ile Ser Gly Cys Arg
85 90 95

Leu Leu Thr Asp Arg Ala Ser Tyr Gly Glu Ala Gln Ala Val Leu Phe
100 105 110

His His Arg Asp Leu Val Lys Gly Pro Pro Asp Trp Pro Pro Pro Trp
115 120 125

Gly Ile Gln Ala His Thr Ala Glu Glu Val Asp Leu Arg Val Leu Asp
130 135 140

Tyr Glu Glu Ala Ala Ala Ala Ala Glu Ala Leu Ala Thr Ser Ser Pro
145 150 155 160

Arg Pro Arg Ala Lys Arg Trp Val Trp Met Asn Phe Glu Ser Pro Ser
165 170 175

His Ser Pro Gly Leu Arg Ser Leu Ala Ser Asn Leu Phe Asn Trp Thr
180 185 190

Leu Ser Tyr Arg Ala Asp Ser Asp Val Phe Val Pro Tyr Gly Tyr Leu
195 200 205

Tyr Pro Arg Ser His Pro Gly Asp Pro Pro Ser Gly Leu Ala Pro Pro
210 215 220

Leu Ser Arg Lys Gln Gly Leu Val Ala Trp Val Val Ser His Trp Asp
225 230 235 240

Glu Arg Gln Ala Arg Val Arg Tyr Tyr His Gln Leu Ser Gln His Val
245 250 255

Thr Val Asp Val Phe Gly Arg Gly Gly Pro Gly Gln Pro Val Pro Glu
260 265 270

Ile Gly Leu Leu His Thr Val Ala Arg Tyr Lys Phe Tyr Leu Ala Phe
275 280 285

Glu Asn Ser Gln His Leu Asp Tyr Ile Thr Glu Lys Leu Trp Arg Asn
290 295 300

Ala Leu Leu Ala Gly Ala Val Pro Val Val Leu Gly Pro Asp Arg Ala
305 310 315 320

Asn Tyr Glu Arg Phe Val Pro Arg Gly Ala Phe Ile His Val Asp Asp
325 330 335

Phe Pro Ser Ala Ser Ser Leu Ala Ser Tyr Leu Leu Phe Leu Asp Arg
340 345 350

Asn Pro Ala Val Val Arg Arg Tyr Phe His Trp Arg Arg Ser Tyr Ala
355 360 365

Val His Ile Thr Ser Phe Trp Asp Glu Pro Trp Cys Arg Val Cys Gln
370 375 380

Ala Val Gln Arg Ala Gly Asp Arg Pro Lys Ser Ile Arg Asn Leu Ala
385 390 395 400

Ser Trp Phe Glu Arg
405

Claims

1. Substantially pure α(1,3) fucosyltransferase comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3 (SEQ ID NO: 2).

2. A fragment or analog of α(1,3)fucosyltransferase polypeptide comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 3 (SEQ ID NO: 2).

3. The polypeptide of claim 1, wherein said polypeptide is derived from a mammal.

4. The polypeptide of claim 3, wherein said cell is of murine or human origin.

5. The polypeptide of claim 4, wherein said murine cell is 32D cl3.

6. Substantially pure DNA having a sequence substantially identical to the nucleotide sequence shown in Fig. 3 (SEQ ID NO: 1).

7. The DNA of claim 6, wherein said DNA is cDNA.

8. A vector comprising the DNA of claim 6.

9. A cell containing the vector of claim 8, said vector being capable of directing expression of the protein encoded by said DNA in a vector-containing cell.

10. A cell which contains the DNA of claim 6.

11. The cell of claim 10, said cell being a eukaryotic cell.

12. The cell of claim 11, wherein said cell is 32D c13 or human cell line 293.

13. A method of fucosylating a polypeptide in vivo comprising:

(a) providing a cell of claim 10; and

(b) culturing said transformed cell under conditions for expressing said DNA, wherein said expression results in the fucosylation of said polypeptide.

14. The method of claim 13, wherein said cell is a human or murine cell.

15. The method of claim 14, wherein said cell is human cell line 293 or a 32D c13 cell.

16. The method of claim 13, wherein said cell contains a second fucosyltransferase gene.

17. The method of claim 16, wherein said second fucosyltransferase gene is substantially identical to the nucleotide sequence shown in Fig. 6A (SEQ ID NO: 3) which encodes a polypeptide comprising an amino acid sequence substantially identical to the amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

18. The method of claim 13, wherein said polypeptide is an antibody or an AGP-antibody fusion protein.

19. A recombinant polypeptide fucosylated using a cell expressing the DNA of claim 6.

20. The polypeptide of claim 19, wherein said fucosylated polypeptide is an antibody or an AGP-antibody fusion protein.

21. The polypeptide of claim 19, wherein said polypeptide is further fucosylated using a second fucosyltransferase.

22. The polypeptide of claim 21, wherein said second fucosyltransferase is substantially identical to a polypeptide comprising an amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

23. A polypeptide fucosylated in vitro using a fucosyltransferase having an amino acid sequence substantially identical to the sequence shown in Fig. 3 (SEQ ID NO: 2).

24. The polypeptide of claim 23, wherein said polypeptide is further fucosylated using a second fucosyltransferase.

25. The polypeptide of claim 24, wherein said second fucosyltransferase comprises an amino acid sequence substantially identical to the sequence shown in Fig. 6B (SEQ ID NO: 4).

26. The polypeptide of claim 23, wherein said fucosylated protein is an AGP-antibody fusion protein.

27. Substantially pure polypeptide of claim 19 or claim 23, wherein said polypeptide is capable of protecting a mammal against an adverse immune reaction.

28. The protein of claim 27, wherein said adverse immune reaction is septic shock or septicemia.

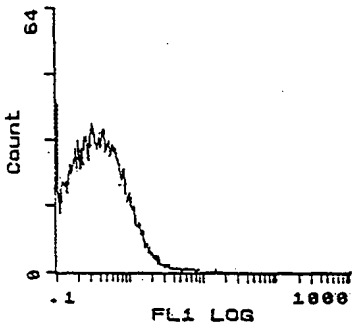
29. A cell containing at least two recombinant fucosyltransferases, one of said fucosyltransferases being substantially identical to the amino acid sequences shown in Fig 3. (SEQ ID NO: 2) and another of said fucosyltransferases being substantially identical to the amino acid sequence shown in Fig. 6B (SEQ ID NO: 4).

30. A method of fucosylating a polypeptide in vitro comprising:

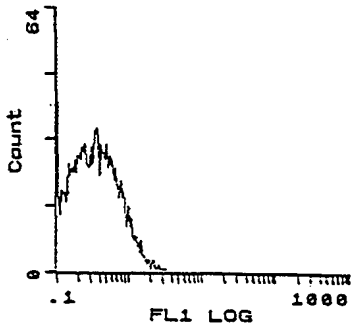
(a) providing an α(1,3) fucosyltransferase of claim 1; and

(b) contacting said polypeptide with said fucosyltransferase under conditions sufficient for fucosylating said polypeptide.

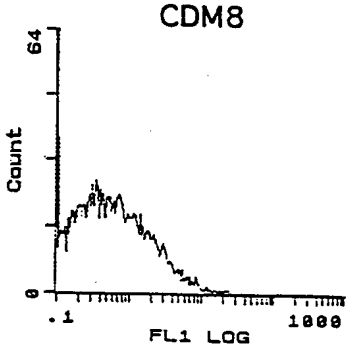
Anti-Lex



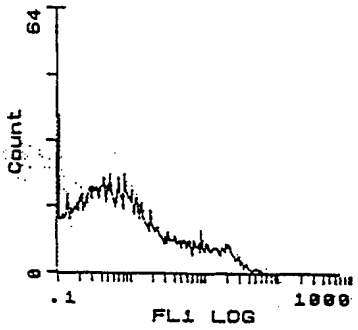
Anti-di/trimeric Lex



Anti-sialyl Lex CDM8



Anti-sialyl Lex



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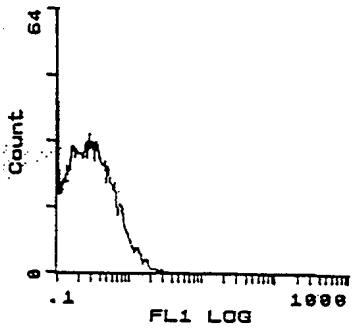

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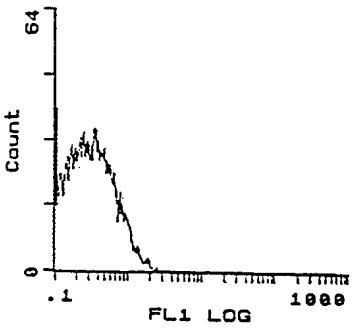

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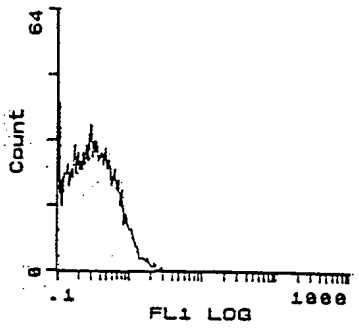
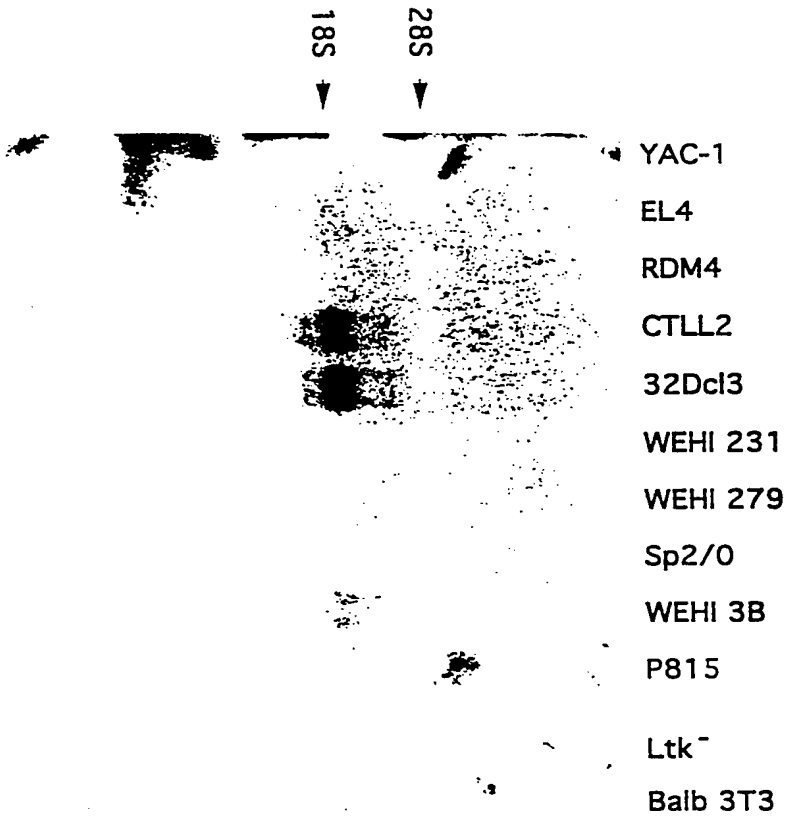


Fig. 1

Fig. 2A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28S
YAC-1
EL4
RDM4
CTLL2
32Dcl3
WEHI 231
WEHI 279
Sp2/0
WEHI 3B
P815
Ltk⁻
Balb 3T3

Fig. 2B

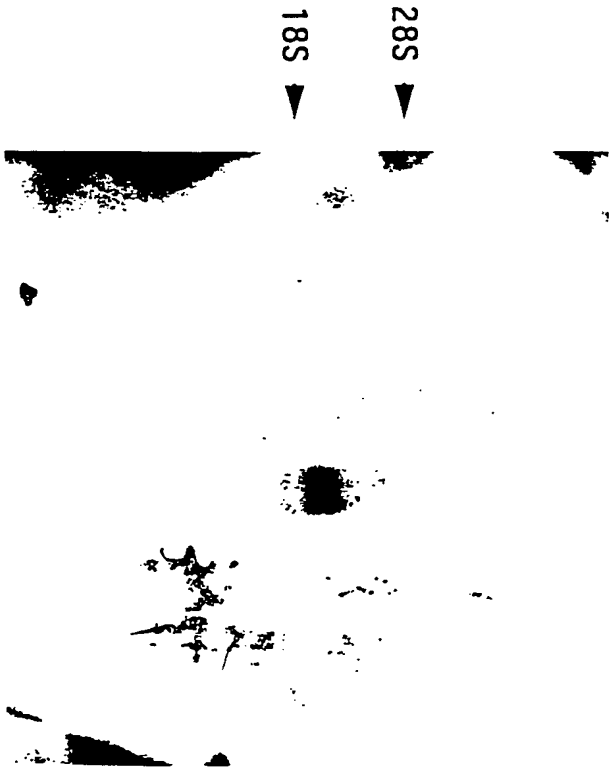
18S
28S
Heart
Sk. muscle
Sm. Intest.
L. Intest.
Stomach
32Dcl3
Liver
Kidneys
Spleen
Brain

Fig. 2C

```
   1  gtagccaaggttcctctccatctcaccagagcctgctggaggggaatcaaacaagcctgg
  61  acctgaggctgggactagctttcctgtttctggagtggatgccaaccccctgcccaccag
 121  cctgcctgtccacgccagggacacacagactccttccctttccagactggaaagccccct
 181  cctgggagagcaggaaggaagcaacctgcaactcttccagccctggaccttgggctgaac
 241  ctacagttcaagggtgcctctgttggagaggctgctgtgatttgaaaatcttctttcctt
 301  ggtgacaattccagaaggctccagatgaattgtattgggtaccaccccaccaggaggctg
                                  M  N  C  I  G  Y  H  P  T  R  R  L
 361  cgggcctggggcggcctagctggaggagcaacattcatggtaatttggtttttctggctg
      R  A  W  G  G  L  A  G  G  A  T  F  M  V  I  W  F  F  W  L
 421  tggggatcagctcctggaagtgcccctgtgcctcagtccacactcaccatccttatctgg
      W  G  S  A  P  G  S  A  P  V  P  Q  S  T  L  T  I  L  I  W
 481  cactggcctttcaccaaccggccgccagagctacctggtgacacctgcactcgctatggc
      H  W  P  F  T  N  R  P  P  E  L  P  G  D  T  C  T  R  Y  G
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 601  ttccaccaccgtgagctgcaaacccggcaatctctcctacccctggaccagaggccacac
      F  H  H  R  E  L  Q  T  R  Q  S  L  L  P  L  D  Q  R  P  H
 661  ggacagccttgggtctgggcctccatggaatcgcccagtaatacccatggtctccatcgc
      G  Q  P  W  V  W  A  S  M  E  S  P  S  N  T  H  G  L  H  R
 721  ttccggggcatcttcaactgggtgctgagctatcggcgtgattcagatatctttgtaCcc
      F  R  G  I  F  N  W  V  L  S  Y  R  R  D  S  D  I  F  V  P
 781  tacggtcgcttggagcctctctctgggcccacatccccactaccggccaaaagcaggatg
      Y  G  R  L  E  P  L  S  G  P  T  S  P  L  P  A  K  S  R  M
 841  gctgcctgggtgatcagcaatttccaggagcggcagcagcgtgcaaagctgtaccggcag
      A  A  W  V  I  S  N  F  Q  E  R  Q  Q  R  A  K  L  Y  R  Q
 901  ctggcccctcatctgcaggtggatgtgttcggtcgcgccagCggacggcccctatgcgct
      L  A  P  H  L  Q  V  D  V  F  G  R  A  S  G  R  P  L  C  A
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1141  cacgtggacgacttcagctctgcccgtgaactggctgtcttcctcgtcagcatgaatgag
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1201  agtcgttatcgtggcttctttgcttggcgagaccggctccgtgtgcggctcctgggtgac
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1261  tggagggagcgcttctgcaccatctgtgcccgctacccttacttgccccgcagccaggtc
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1321  tatgaagaccttgaaagctggttccaggcttgaactcctgctgctgggagaggctggatg
      Y  E  D  L  E  S  W  F  Q  A  *
1381  ggtgggagactgatgttgaaaccaaagagctgggcatccaggcttttggtcaccatggca
1441  ctaccccaaggcttttcctgttcagtgagcaggaattcaggatataaggagaaaactggg
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1801  ctactaataaaaac  1814
```

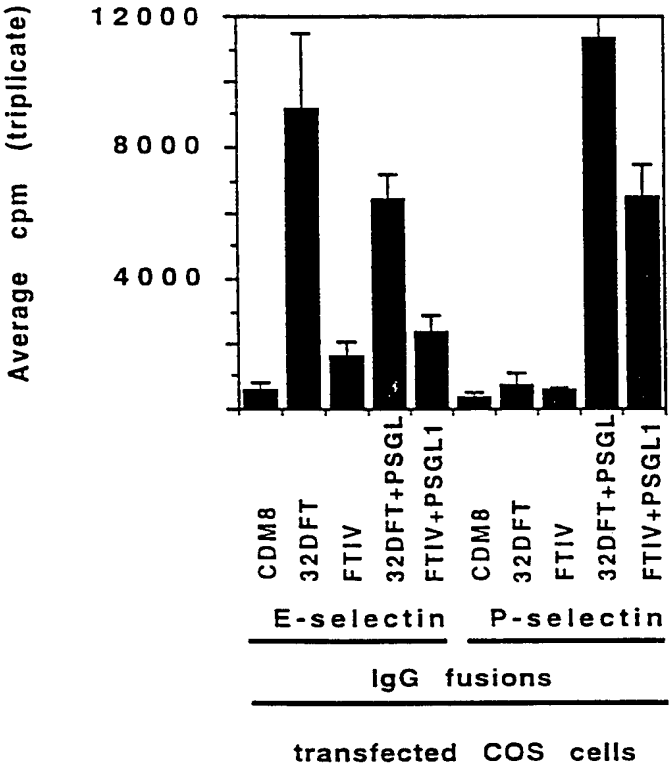
Fig. 3

(SEQ ID NOS: 1, 2)

Anti-Lex
Anti-sialyl Lex
Anti-CD65
Anti-di/trimeric Lex
Anti-mouse IgM ctrl
Anti-mouse IgG ctrl
32D cl3
32D FT3IV (myeloid)
Count
FL1 LOG
0
.1
1000
38
86
64
42
34
36
71

Fig. 4

Fig. 5 A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4000
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32DFT
FTIV
32DFT+PSGL
FTIV+PSGL1
CDM8
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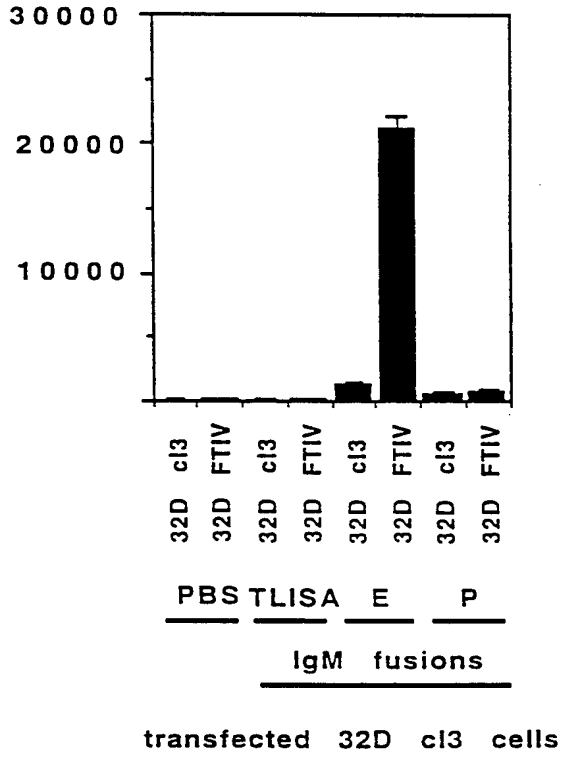
FTIV
32DFT+PSGL
FTIV+PSGL1
E-selectin
P-selectin
IgG fusions
transfected COS cells

Fig. 5 B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20000
10000
32D cl3
32D FTIV
32D cl3
32D FTIV
32D cl3
32D FTIV
32D cl3
32D FTIV
PBS
TLISA
E
P
IgM fusions
transfected 32D cl3 cells

Fig. 6A

SEQ ID NO: 3

```
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  51  GGCGGCGGGC GGGCGGCGCG GGTGGCGCCG AGGCCGGGGG CTGCCATGGA
 101  CCGTCTGTGT GCTGGCGGCC GCCGGCTTGA CGTGTACGGC GCTGATCACC
 151  TACGCTTGCT GGGGGCAGCT GCCGCCGCTG CCTGGGCGTC GCCAACCCCG
 201  TCGCGACCGG TGGGCGTGCT GCTGTGGTGG GAGCCCTTCG GGGGCGCGAT
 251  CAGCGCCCCG AGGCCGCCCC CTGACTGCCG GCTGCGCTTC AACATCAGCG
 301  GCTGCCGCCT GCTCACCGAC ACGCGCGTCC TACGGAGAGG CTCAGGCCGT
 351  GCTTTTCCAC CACCGCGACC TCGTGAAGGG GCCCCCCGAC TGGCCCCCGC
 401  CCTGGGGCAT CCAGGCGCAC ACTGCCGAGG AGGTGGATCT GCGCGTGTTG
 451  GACTACGAGG AGGCAGCGGC GGCGGCAGAA GCCCTGGCGA CCTCCAGCCC
 501  CAGGCCCCGG GCCAAGCGCT GGGTTTGGAT GAACTTCGAG TCGCCCTCGC
 551  ACTCCCCGGG GCTGCGAAGC CTGGCAAGTA ACCTCTTCAA CTGGACGCTC
 601  TCCTACCGGG CGGACTCGGA CGTCTTTGTG CCTTATGGCT ACCTCTACCC
 651  CAGAAGCCAC CCCGGCGACC CGCCCTCAGG CCTGGCCCCG CCACTGTCCA
 701  GGAAACAGGG GCTGGTGGCA TGGGTGGTGA GCCACTGGGA CGAGCGCCAG
 751  GCCCGGGTCC GCTACTACCA CCAACTGAGC CAACATGTGA CCGTGGACGT
 801  GTTCGGCCGG GGCGGGCCGG GGCAGCCGGT GCCCGAAATT GGGCTCCTGC
 851  ACACAGTGGC CCGCTACAAG TTCTACCTGG CTTTCGAGAA CTCGCAGCAC
 901  CTGGATTATA TCACCGAGAA GCTCTGGCGC AACGCGTTGC TCGCTGGGGC
 951  GGTGCCGGTG GTGCTGGGCC CAGACCGTGC CAACTACGAG CGCTTTGTGC
1001  CCCGCGGCGC CTTCATCCAC GTGGACGACT TCCCAAGTGC CTCCTCCCTG
1051  GCCTCGTACC TGCTTTTCCT CGACCGCAAC CCCGCGGTCT ATCGCCGCTA
1101  CTTCCACTGG CGCCGGAGCT ACGCTGTCCA CATCACCTCC TTCTGGGACG
1151  AGCCTTGGTG CCGGGTGTGC CAGGCTGTAC AGAGGGCTGG GACCGGCCCA
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1551  CGTTGAGGCG GTTTCCTGAA TTTCCCCATC TGCCACAGGC CATATTTGTG
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1651  TCTGGACCAA GGTGAAGCAA ATTTGTGGTT GTAGAAGGAG CCTTGTTGGT
1701  GGAGAGTGGA AGGACTGTGG CTGCAGGTGG GACTTTGTTG TTTGGATTCC
1751  TCACAGCCTT GGCTCCTGAG AAAGGTGAGG AGGGCAGTCC AAGAGGGGCC
1801  GCTGACTTCT TTCACAAGTA CTATCTGTTC CCCTGTCCTG TGAATGGAAG
1851  CAAAGTGCTG GATTGTCCTT GGAGGAAACT TAAGATGAAT ACATGCGTGT
1901  ACCTCACTTT ACATAAGAAA TGTATTCCTG AAAAGCTGCA TTTAAATCAA
1951  GTCCCAAATT CATTGACTTA GGGGAGTTCA GTATTTAATG AAACCCTATG
2001  GAGAATTTAT CCCTTTACAA TGTGAATAGT CATCTCCTAA TTTGTTTCTT
2051  CTGTCTTTAT GTTTTTCTAT AACCTGGATT TTTTAAATCA TATTAAAATT
2101  ACAGATGTGA AAATAAAAAA AAAAAAAAAA AAAA
```

Fig. 6A

SEQ ID NO: 4

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  1  MGAPWGSPTA AAGGRRGWRR GRGLPWTVCV LAAAGLTCTA LITYACWGQL
 51  PPLPWASPTP SRPVGVLLWW EPFGGAISAP RPPPDCRLRF NISGCRLLTD
101  RASYGEAQAV LFHHRDLVKG PPDWPPPWGI QAHTAEEVDL RVLDYEEAAA
151  AAEALATSSP RPRAKRWVWM NFESPSHSPG LRSLASNLFN WTLSYRADSD
201  VFVPYGYLYP RSHPGDPPSG LAPPLSRKQG LVAWVVSHWD ERQARVRYYH
251  QLSQHVTVDV FGRGGPGQPV PEIGLLHTVA RYKFYLAFEN SQHLDYITEK
301  LWRNALLAGA VPVVLGPDRA NYERFVPRGA FIHVDDFPSA SSLASYLLFL
351  DRNPAVYRRY FHWRRSYAVH ITSFWDEPWC RVCQAVQRAG DRPKSIRNLA
401  SWFER*
```

Fig. 6 B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : C12N 9/10, 9/64; C12P 21/06
US CL : 435/226, 193, 69.1,
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/226, 193, 69.1,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN (medline,caplus, wpids). Search terms: Fucosyltransferase; Fucosyltransferase and amino acid sequence, .. and DNA.. and murine ... and Human.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal Biol. Chem.,Volume 269, Number 20, issued 20 May, 1994, Sasaki et al., "Expression Cloning of a Novel alpha1,3-Fucosyltransferase that is Involved in the Biosynthesis of Sialyl Lewis X Carbohydrate", pages 14730-14737. 81.3% query match in protein database search - MasPar. Nucleotide and deduced amino acid sequence of a novel 1,3-fucosyltransferase, see Fig 2., page 14734.	1-5 and 30

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 19 JULY 1996	Date of mailing of the international search report 19 AUG 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Crouch Tekchand Saidha Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Journal Biol. Chem., Volume 269, Number 24, Issued 17 June, 1994, Natsuka et al., "Molecular Cloning of a cDNA Encoding a Novel Human Leukocyte alpha 1,3-Fucosyltransferase Capable of Synthesizing the Sialyl Lewis x Determinant", pages 16789-16794. Novel DNA and Amino acid sequences of human leukocyte alpha 1,3-fucosyltransferase, see Fig.1., page 16791. Protein sequence query match - 79.7%.	1-5 and 30
X	Journal Biol. Chem. Volume 267, Number 34, Issued 05 December , 1992, Weston et al., " Molecular cloning of a Forth Member of a Human alpha 1, 3- fucosyltransferase gene", pages 24575-24584, Fig.1. DNA and protein sequences are disclosed. Fig. 2, shows four cloned human alpha 1,3-fucosyltransferase.	1-5 and 30
Y	Journal Biol. Chem. Volume 269, Number 2, Issued 14 January , 1994, Goelz et al., "Differential Expression of an E-selectin Ligand .... alpha 1,3- Fucosyltransferase Gene (ELET)", pages 1033-1040.	30
X	WO, A, 94/23021 (KYOW HUKKO KOOYO KK) 13 October 1994, page 1, abstract.	1-5 and 30

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 and 30

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/06427

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1-5 and 30, drawn to a fucosyltransferase "substantially identical" to SEQ ID NO:2.
Group II, claims 6-18 and 29, drawn to DNA "substantially identical" to SEQ ID NO:1, vectors and host cells comprising said DNA and a method of using said DNA.
Group III, claims 19, 20, 23, and 26-28, drawn to a fucosylated protein.
Group IV, claims 21, 22, 24, and 25, drawn to a doubly fucosylated protein.

The inventions listed as Groups I-IV do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The claims of Group I share a technical feature of a fucosyltransferase "substantially identical" to the amino acid sequence of SEQ ID NO:2 (i.e., sharing at least 50% sequence identity withn SEQ ID NO:2, see page 5, lines 15-30 of the disclosure). The claims of Group II share a technical feature of DNA "substantially identical" to SEQ ID NO:1. The claims of Group III share a technical feature of fucosylation and the claims of Group IV share a technical feature of double fucosylation.

Fucosyltransferases sharing at least 50% sequence identity with, and therefore "substantially identical" to, SEQ ID NO:2 were known in the prior art. See Sasaki et al. (Journal of Biological Chemistry, Vol. 269, No. 20, issued 20 May 1994, pages 14730-14737) and Natsuka et al. (Journal of BiologicalChemistry,Vol.269, No. 24, issued 17 June 1994, pages 16789-16794). In addition, fucosylated and multiply fucosylated proteins were known in the art (see, e.g., Sasaki et al., Figure 5 and Table II, and Natsuka et al., Figure 3 and Table II). The various Groups of inventions thus do not share a technical relationship involving one or more of the same or corresponding special technical features, i.e., those technical features that define a contribution which each invention, considered as a whole, makes over the prior art. They therefore do not fulfill the requirements of unity of invention and a holding of lack of unity for examination purposes is proper.